IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Board of Regents, the University of Texas System	)	
Serial No.: PCT/US03/23131	)	
Filed: 24 July 2003 (24.07.03)	)	Atty. Dkt.: 5119-11101
For: Capture and Detection of Microbes by Membrane Methods	)	

"EXPRESS MAIL" MAILING LABEL NUMBER EV325433898US DATE OF DEPOSIT 26 APRIL 2004 (26.04.04). I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE - "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 C.F.R. § 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE: COMMISSIONER FOR PATENTS, M/S PCT, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450

Derrick Brown

RESPONSE TO WRITTEN OPINION
MAILED 24 FEBRUARY 2004 (24.02.04)

Commissioner for Patents
Mail Stop PCT
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sirs:

Amendment

Please amend the above-captioned application as follows:

In the Claims:

Please cancel claim 13 without prejudice.

The following listing of claims will replace all prior versions and listings of claims in the application:

1. (currently amended) An analyte detection device comprising:

a body;

a porous membrane coupled to the body;

a membrane support in contact with the membrane, wherein the membrane support is configured to maintain the membrane in a substantially planar orientation during use;

a top member positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light, and

wherein the top member comprises a fluid inlet configured to allow fluid to be introduced to the membrane through the top member, and wherein the top member comprises a wash fluid outlet configured to allow fluid to pass across the membrane out of the analyte detection device during a washing operation; and

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use.

2. (original) The device of claim 1, wherein the porous membrane comprises pores having a diameter between about 0.2 microns to about 12 microns.

3. (original) The device of claim 1, wherein the top member is substantially transparent to visible light.

4. (original) The device of claim 1, wherein the bottom member is substantially transparent to visible light.

5. (original) The device of claim 1, wherein the top member is substantially transparent to visible light, and wherein the bottom member is substantially transparent to visible light.

6. (original) The device of claim 1, wherein the top member is substantially transparent to ultraviolet light.

7. (original) The device of claim 1, wherein the bottom member is substantially transparent to ultraviolet light.

8. (original) The device of claim 1, wherein the top member is substantially transparent to ultraviolet light, and wherein the bottom member is substantially transparent to ultraviolet light.

9. (currently amended) The device of claim 1, wherein the bottom member comprises an indentation configured ~~t~~to receive the membrane.

10. (original) The device of claim 1, wherein the bottom member comprises a first indentation configured to receive the filter and a second indentation which is configured to receive fluid passing through the membrane during use.

11. (original) The device of claim 1, ~~further comprising a membrane support coupled to the membrane,~~ wherein the membrane support is composed of a porous material.

12. (original) The device of claim ~~11~~1, wherein the membrane support comprises pores that allow fluid to flow through the membrane support at a speed that is equal to or greater than the speed that fluid passes through membrane.

13. (original) The device of claim ~~11~~1, wherein the membrane support provides sufficient support of the membrane during use to inhibit sagging of the membrane.

14. (canceled)

15. (original) The device of claim 1, further comprising a gasket positioned between the membrane and the top member.

16. (original) The device of claim 1, wherein the top member comprises a fluid inlet configured to allow fluid to be introduced to the membrane through the top member.

17. (original) The device of claim 1, wherein the bottom member comprises a fluid outlet configured to allow fluid to pass from the membrane out of the analyte detection device.

18. (canceled)

19. (original) The device of claim 1, further comprising a body and a cap, wherein the top member, the membrane and the bottom member are disposed within the body, and wherein the cap secures the top member, the membrane and the bottom member within the body.

20. (original) The device of claim 1, wherein top member is composed of an acrylate polymer.

21. (original) The device of claim 1, wherein the top member and the bottom member are composed of an acrylate polymer.

22. (currently amended) An analyte detection system comprising:

an analyte detection device, the analyte detection device comprising:
a body;
a porous membrane coupled to the body;
a membrane support in contact with the membrane, wherein the membrane support is configured to maintain the membrane in a substantially planar orientation during use;
a top member positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light, and wherein the top member comprises a fluid inlet configured to allow fluid to be introduced to the membrane through the top member, and wherein the top member comprises a wash fluid outlet configured to allow fluid to pass across the membrane out of the analyte detection device during a washing operation; and

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use

a detector optically coupled to the porous membrane, wherein the detector is configured to view at least a portion of the membrane through the window;

a fluid delivery system coupled to the analyte detection device, wherein the fluid delivery system is configured to deliver fluid samples to the analyte detection device.

23. (original) The system of claim 22, wherein the detector comprises a CCD camera.

24. (original) The system of claim 22, wherein the fluid delivery system comprise one or more pumps.

25. (original) The system of claim 22, wherein the fluid delivery system comprises a plurality of pumps, each of the pumps coupled to a different fluid storage container.

26. (original) The system of claim 22, wherein the fluid delivery system comprises one or pumps and one or more filters, wherein the filters are configured to filter fluids before the fluids are delivered to the analyte detection device.

27. (original) The system of claim 22, wherein the analyte detection device comprises a fluid inlet, and wherein the fluid delivery system comprises a plurality of pumps each of the pumps coupled to a different fluid storage container and a manifold, wherein the manifold is configured to redirect fluid from received from at least a portion of the pumps to the fluid inlet.

28. (original) The system of claim 22, wherein the detector comprises a microscope.

29. (original) The system of claim 22, further comprising a programmable controller coupled to the fluid delivery system.

30. (original) The system of claim 29, wherein the programmable controller is further coupled to the detector.

31. (currently amended) A method of sensing an analyte in a fluid comprising:

passing the fluid across a porous membrane disposed in an analyte detection device configured to capture the analyte on the porous membrane, wherein the analyte detection device comprises:

a body;

a porous membrane coupled to the body;

a membrane support in contact with the membrane, wherein the membrane support is configured to maintain the membrane in a substantially planar orientation during use;

a top member positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, wherein the top member

covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light, and

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

detecting an image of matter captured on the porous membrane; and

determining if the analyte is present on the porous membrane.

32. (original) The method of claim 31, further comprising passing the analyte to a sensor array if the image meets the user-defined criteria.

33. (original) The method of claim 31, wherein the sensor array comprises a porous particle.

34. (original) The method of claim 31, wherein determining if the analyte is present comprises comparing the shape of the matter to user-defined criteria.

35. (original) The method of claim 31, determining if the analyte is present comprises comparing the size of the matter to user-defined criteria.

36. (original) The method of claim 31, determining if the analyte is present comprises comparing the aggregate area of the matter to user-defined criteria.

37. (original) The method of claim 31, determining if the analyte is present comprises comparing the color of the matter to user-defined criteria.

38. (original) The method of claim 31, determining if the analyte is present comprises comparing the fluorescence of the matter to user-defined criteria.

39. (original) The method of claim 31, determining if the analyte is present comprises comparing the fluorescent intensity of the matter to user-defined criteria.

40. (original) The method of claim 31, further comprising applying a stain to the matter captured on the membrane.

41. (original) The method of claim 31, further comprising collecting a sample of an analyte in a fluid using an air collection device.

42. (original) The method of claim 31, further comprising passing a background fluid through the filter and detecting an image captured on the porous membrane prior to passing the fluid containing the analyte across the porous membrane.

43. (original) The method of claim 31, further comprising performing a lateral flush to clean the surface of the membrane prior to passing the fluid containing the analyte across the membrane.

44. (original) The method of claim 31, further comprising performing a back flush to clean the surface of the membrane prior to passing the fluid containing the analyte across the membrane.

45. (original) The method of claim 31, wherein detecting an image is performed using a CCD detector.

46. (original) The method of claim 31, wherein detecting an image is performed using a microscope.

47. (original) The method of claim 31, further comprising further comprising passing a visualization agent across the membrane after the fluid is passed over the membrane.

48. (original) The method of claim 31, further comprising performing a lateral wash of the membrane after detecting an image.

49. (original) The method of claim 31, further comprising performing a back wash of the membrane after detecting an image.

50. (original) A method of analyzing an analyte collected on a membrane comprising:

passing a fluid sample across a membrane, wherein the fluid sample comprises an analyte that is at least partially retained by the membrane;

adding a visualization agent to material collected on the membrane when the fluid sample is passed across the membrane;

collecting an image of the collected material using white light, at a first wavelength of light, a second wavelength of light, and a third wavelength of light, wherein the analyte comprises a color corresponding to the first wavelength of light;

forming a first mask corresponding to an image of the collected material at the second wavelength of light;

forming a second mask corresponding to an image of the collected material at the third wavelength of light;

subtracting the first mask and the second mask from the image of the collected material in white light.

51. (original) The method of claim 50, wherein the wavelengths of light are selected from the group consisting of red, blue and green.

52. (original) The method of claim 50, wherein the collecting the image data and forming the masks is performed by a computer.

53. (original) The method of claim 50, further comprising determining the amount of analyte present on the membrane by analysis of the image resulting from subtracting the first mask and the second mask from the image of the collected material in white light.

54. (original) The method of claim 50, wherein the images are collected using a CCD detector.

55. (original) The method of claim 50, wherein the images are collected using a CCD detector coupled to a microscope.

56. (original) A particle for detecting an analyte in a fluid comprising a receptor coupled to a polymeric resin, wherein the polymeric resin comprises a plurality of pores having a diameter of less than about 1 μm.

57. (original) The particle of claim 56, further comprising a receptor coupled to the surface of one of the pores.

58. (original) The particle of claim 56, wherein the particle comprises agarose.

59. (original) The particle of claim 56, wherein the particle is substantially spherical.

60. (original) The particle of claim 56, wherein the particle has a diameter of between about 100 to 500 microns.

61. (original) The particle of claim 56, wherein the particle is configured to entrap microbes.

62. (original) A method for forming a porous particle, comprising:

forming an emulsion of a polymeric resin in an aqueous solution;

reducing the temperature of the emulsion to produce the porous particle, wherein the porous particle comprises a plurality of pores having a diameter of less than about 1 μm.

63. (original) The method of claim 62, wherein forming an emulsion comprises adding an emulsifier to a mixture of the polymeric resin in water.

64. (original) A porous particle, formed by the method of claim 62.

65. (currently amended) A method for detecting a microbe, comprising:

passing the fluid over a porous particle configured to capture the microbe, wherein the porous particle comprises a plurality of pores having a diameter of less than about 1 μm; and

detecting the microbe with a detector.

66. (original) The method of claim 65, wherein a receptor configured to receive the microbe is coupled to the porous particle.

67. (original) A system for detecting an analyte in a fluid comprising:

a light source;

a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member;

a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use, and wherein the particle comprises a receptor coupled to a polymeric resin, wherein the polymeric resin comprises a plurality of pores having a diameter of less than about 1 μm; and

a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;

wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

68. (original) A method of sensing an analyte in a fluid comprising:

passing a fluid over a sensor array, the sensor array comprising at least one particle positioned within a cavity of a supporting member, wherein the particle comprises a receptor coupled to a polymeric resin, wherein the polymeric resin comprises a plurality of pores having a diameter of less than about 1 μm;

monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

69. (currently amended) A method of sensing an analyte in a fluid comprising:

passing the fluid across a porous membrane configured to capture the analyte on the porous membrane;

applying a visualization agent to the particles on the porous membrane;

detecting an image of matter captured on the porous membrane with a detector at a plurality of wavelengths of light;

detecting an image of matter captured on the porous membrane at a specific wavelength ~~pf~~ of light, wherein the specific wavelength of light represents light that is not indicative of the presence of the analyte.

70. (original) A method of forming a sensor array comprising:

forming a plurality of cavities in a supporting member;

forming a plurality of particles, wherein each particle comprises a receptor coupled to a polymeric resin, wherein a plurality of different receptors are coupled to the particles;

interacting the plurality of particles with an analyte;

determining which particles interact with the analyte and the extent to which the interact with the analyte;

separating particles that interact with the analyte and meet a predetermined criteria from particles that do not substantially interact with the analyte or do not meet a predetermined criteria;

adding the separated particles that interact with the analyte and meet the predetermined criteria to a sensor array.

71. (original) The method of claim 70, wherein separating the particles comprises separating the particles using a flow cytometer.

72. (canceled)

73. (cancelled)

74. (new) An analyte detection system comprising:

an analyte detection device, the analyte detection device comprising:

a body;

a porous membrane coupled to the body;

a top member, wherein the top member comprises a fluid inlet configured to allow fluid be introduced to the membrane through the top member, and wherein the top member is positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, and wherein the top member covers at

least a portion of the porous membrane, and wherein the top member is substantially transparent to light;

a membrane support in contact with the membrane, wherein the membrane support is configured to maintain the membrane in a substantially planar orientation during use;

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

a detector optically coupled to the porous membrane, wherein the detector is configured to view at least a portion of the membrane through the window;

a fluid delivery system coupled to the analyte detection device, wherein the fluid delivery system is configured to deliver fluid samples to the analyte detection device.

75. (new) An analyte detection system comprising:

an analyte detection device, the analyte detection device comprising:

a body;

a porous membrane coupled to the body;

a top member, wherein the top member comprises a fluid inlet configured to allow fluid be introduced to the membrane through the top member, and wherein the top member is positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, and wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light;

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

a detector optically coupled to the porous membrane, wherein the detector is configured to view at least a portion of the membrane through the window;

a fluid delivery system coupled to the analyte detection device, wherein the fluid delivery system is configured to deliver fluid samples to the analyte detection device, and wherein the fluid delivery system comprises one or more pumps, each of the pumps coupled to a different fluid storage container.

76. (new) An analyte detection system comprising:

an analyte detection device, the analyte detection device comprising:

a body;

a porous membrane coupled to the body;

a top member, wherein the top member comprises a fluid inlet configured to allow fluid be introduced to the membrane through the top member, and wherein the top member is positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, and wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light;

a membrane support in contact with the membrane, wherein the membrane support is configured to maintain the membrane in a substantially planar orientation during use;

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

a detector optically coupled to the porous membrane, wherein the detector is configured to view at least a portion of the membrane through the window;

a fluid delivery system coupled to the analyte detection device, wherein the fluid delivery system is configured to deliver fluid samples to the analyte detection device, and wherein the fluid delivery system comprises a one or more pumps and one or more filters, wherein the filters are configured to filter fluids before the fluids are delivered to the analyte detection device.

77. (new) An analyte detection system comprising:

an analyte detection device, the analyte detection device comprising:

a body;

a porous membrane coupled to the body;

a top member, wherein the top member comprises a fluid inlet configured to allow fluid be introduced to the membrane through the top member, and wherein the top member is positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, and wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light;

a membrane support in contact with the membrane, wherein the membrane support is configured to maintain the membrane in a substantially planar orientation during use;

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

a detector optically coupled to the porous membrane, wherein the detector is configured to view at least a portion of the membrane through the window; and

a fluid delivery system coupled to the analyte detection device, wherein the fluid delivery system is configured to deliver fluid samples to the analyte detection device, and wherein the fluid delivery system comprises a one or more pumps each of the pumps coupled to a different fluid storage container and a manifold, wherein the manifold is configured to redirect fluid received from one or more pumps toward the fluid inlet.

78. (new) A method of sensing an analyte in a fluid comprising:

passing the fluid across a porous membrane disposed in an analyte detection device configured to capture the analyte on the porous membrane, wherein the analyte detection device comprises:

a body;

a porous membrane coupled to the body;

a top member positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light, and

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

applying a visualization agent to the matter captured on the porous membrane

detecting an image of matter captured on the porous membrane; and

determining if the analyte is present on the porous membrane.

79. (New) A method of sensing an analyte in a fluid comprising:

passing a background fluid across a porous membrane disposed in an analyte detection device, wherein the analyte detection device comprises:

a body;

a porous membrane coupled to the body;

a top member positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light, and

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

detecting an image of matter captured on the porous membrane after passing the background fluid through the porous membrane;

cleaning the surface of the porous membrane

passing a fluid containing one or more analytes across the porous membrane;

detecting an image of the matter captured on the porous membrane after passing the fluid containing one or more analytes through the membrane; and

determining if the analyte is present on the porous membrane by comparing the image of matter captured on the porous membrane after passing the fluid containing one or more analytes through the membrane to the image of matter captured on the porous membrane after passing the background fluid through the porous membrane.

80. (New) A method of sensing an analyte in a fluid comprising:

passing a first sample fluid across a porous membrane disposed in an analyte detection device, wherein the analyte detection device comprises:

a body;

a porous membrane coupled to the body;

a top member positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top member and the porous membrane, wherein the top member covers at least a portion of the porous membrane, and wherein the top member is substantially transparent to light, and

a bottom member positioned below the porous membrane, wherein the bottom member is configured to receive fluid flowing through the porous membrane during use;

detecting an image of matter captured on the porous membrane after passing the first fluid sample through the porous membrane;

determining if an analyte is present in the first fluid sample;

cleaning the surface of the porous membrane

passing a second sample fluid across the porous membrane;

detecting an image of matter captured on the porous membrane after passing the second fluid sample through the membrane; and

determining if an analyte is present in the second fluid sample.

Response To Written Opinion Mailed February 24, 2004

A. Claims in the Case

Claim 14 has been cancelled. Claims 1, 9, 11-13, 18, 22, 31, 65, and 69 have been amended. Claims 74-80 have been added. Claims 1-13 and 15-80 are pending.

B. The Claims Are Not Anticipated by Rotman

The Written Opinion states that claims 1-30 and 72 lacked novelty as being anticipated by U.S. Patent No. 4,734,372 to Rotman ("Rotman"). Applicant respectfully disagrees that the claims lack novelty.

The Written Opinion states that Rotman discloses an analyte detection system that includes a body, porous membrane, a top member which is transparent to light, and a cavity.

Amended claim 1 and 22 include a combination of features including, but not limited to, the features of "a membrane support coupled to the membrane." Rotman does not appear to teach or suggest at least the quoted features of the claim.

Rotman teaches:

> [w]ithin the cavity of the shell elements **12,14**, a cell compartment **20** is formed by a hollow cylindrical element 18 and porous upper and lower membranes **22, 24**, respectively. The edges of the membranes **22,24**, and the hollow cylindrical element **18** are sealed to the shell by upper and lower elastomeric O-ring gaskets **26,28**, respectively. (Rotman, column 5, lines 24-30)

Rotman appears to teach a two membrane system that is sealed to a compartment with O-rings. Rotman does not appear to teach or suggest a membrane support coupled to the membrane. Applicant submits that independent claims 1 and 22 and dependent claims 2-21and 23-30 are novel since Rotman does not appear to teach all the features of the claims.

C. The Claims Are Not Anticipated by Freiburghaus et al.

The Written Opinion states that claims 56-61 and 64 lacked novelty as being anticipated by European Patent No. 0 109 531 to Freiburghaus et al. ("Freiburghaus"). Applicant respectfully disagrees that the claims lack novelty.

The Written Opinion states that Freiburghaus discloses an analyte detection particle that includes a receptor and a plurality of pores that have a diameter of less than 1 micron, citing pages 5 and 6 of Freiburghaus.

Freiburghaus states:

> It is particularly advantageous in accordance with the invention to use porous macromolecular gel particles as a carrier for the immobilized antigens, since such gel particles present suitable flow conditions for the body fluid and make it possible in fact to carry out the intended treatment directly on blood.
>
> Such macromolecular carriers in accordance with the invention can be appropriately gel particles containing agarose (Pharmacia Fine Chemicals AB, Upsala, Sweden). Gel particles containing agarose of the type Sepharose 6MB of a particle size of 200 – 300 μm have been found to be particularly suitable."
> (Freiburghaus, page 5, lines 19-28)

Freiburghaus appears to teach the use of porous agrose particles. Freiburghaus, however, does not appear to teach the specific pore size of the particles. Based on the cited particle size of 200 - 300 μm, Applicant submits that the particles described by Freiburghaus may be greater than 1 μm. In the absence of any specific teaching of pore size, Applicant respectfully requests removal of this rejection.

Furthermore, Freiburghaus states that:

> The antigens are in fact immobilized on a carrier 20 in such a manner that the mutual distance between the active binding sites of the immobilized antigens in the main is greater than the distance between the two Fab-regions of the actual antibody.
>
> Since the maximum distance between the two Fab-regions of an antibody normally amounts to approx. 140 Å, this means that the immobilized antigens in the column 1 are situated at a distance from one another of at least 140 Å.
> (Freiburghaus, pg. 5, lines 1-8)

Freiburghaus appears to teach that the antibodies are placed on a support such that the distance between two antibodies is at least 140 Å. Freiburghaus does not appear to teach any specific reference to pore size in the quoted section.

Applicant submits that Freiburghaus does not appear to teach or suggest at least the quoted features of claim 56. Applicant submits that independent claim 56 and dependent claims 57-61 are novel with regard to Freiburghaus.

D. <u>The Claims Are Not Anticipated by Poitras</u>

The Written Opinion states that claims 1-17, 19-21 and 72 lacked novelty as being anticipated by U.S. Patent No. 2,923,669 to Poitras ("Poitras"). Applicant respectfully disagrees that the claims lack novelty.

The Written Opinion states that Poitras disclosed an analyte detection system that includes a body, a porous membrane, a top member that is transparent to light, and a cavity.

Amended claim 1 and 22 include a combination of features including, but not limited to, the feature of a "top member comprises a fluid inlet configured to allow fluid to be introduced to the membrane through the top

member, and wherein the top member comprises a wash fluid outlet configured to allow fluid to pass across the membrane out of the analyte detection device during a washing operation."

Applicant submits that Poitras does not appear to teach or suggest at least this feature of claims 1 and 22.

E. The Claims Are Not Obvious Over Weinreb et al.

The Written Opinion states that claims 1-49 lacked inventive step as being obvious over U.S. Patent No. 5,506,141 to Weinreb et al. ("Weinreb"). Applicant respectfully disagrees that the claims lack an inventive step.

The Written Opinion states that Weinreb discloses an analyte detection system and method of use that includes a body for supporting a porous membrane.

Amended claims 1, 22 and 31 each include a combination of features including, but not limited to, the features of "a membrane support coupled to the membrane." Applicant submits that Weinreb does not appear to teach or suggest at least the quoted features of the claim.

Referring to FIG. 11 of Weinreb, Applicant submits that the system of Weinreb does not appear to teach or suggest the use of a membrane support. Specifically, the "cell carrier" of Weinreb (depicted as a thin, cross-hatched rectangle over opening 56, unnumbered) appears to be disposed over an opening. None of the figures of Weinreb appear to teach or suggest the presence of a membrane support. Applicant submits, for at least these reasons, that claims 1, 22, and 31 are patentable over Weinreb.

F. The Claims Are Not Obvious Over Freiburghaus in view of Li et al.

The Written Opinion states that claims 62-64 lack an inventive step as being obvious over Freiburghaus et al in view of U.S. Patent No. 5,863,957 to Li et al. ("Li"). Applicant respectfully disagrees that the claims lack an inventive step.

The Written Opinion states "Freiburghaus has been discussed above. While the reference discloses a porous analyte bead, the reference is silent as to the method of making the bead." The Written Opinion also states that Li teaches forming porous gel beads by forming an emulsion and controlling the temperature.

Claim 62 includes a combination of features including, but not limited to, the features of "reducing the temperature of the emulsion to produce the porous particle."

For at least the reasons cited above, Freiburghaus does not appear to teach or suggest a particle having the properties of Applicant's claims, including, but not limited to, a particle comprising "a plurality of pores

having a diameter of less than about 1 μm." Applicant further submits that the process of making the claimed particle is not taught or suggested by Li, or the combination of Li and Freiburghaus. Li states:

> The present invention also relates to a process for producing a porous, crosslinked polymeric microbead as well as the product of this process. This process involves combining an oil phase with an aqueous discontinuous phase to form an emulsion, adding the emulsion to an aqueous suspension medium to form an oil-in-water suspension of dispersed emulsion droplets, and polymerizing the emulsion droplets to form microbeads.
> (Li, Abstract)

Li appears to be directed to a technique of forming beads by emulsion polymerization. Specifically, Li appears to teach that a monomer is dispersed as an aqueous medium to produce an emulsion. This emulsion is polymerized by heating the mixture.

Applicant's claim 62 recites, in part:

> forming an emulsion of a polymeric resin in an aqueous solution;
> reducing the temperature of the emulsion to produce the porous particle, wherein the porous particle comprises a plurality of pores having a diameter of less than about 1 μm.

Applicant's process is directed to forming a particle from a water-in-oil emulsion of a polymer, not a monomer. For example, Applicant's specification states:

> A dispersion of a hydrophilic emulsifier (such as TWEEN 85) in cyclohexane may be added to a stirring aqueous solution of agarose at 60°C for 5 min to produce an oil-in-water emulsion. Fine particles of agarose stabilized by the emulsifier may be formed in this step. Next, a solution of a hydrophobic emulsifier (such as SPAN 85) may be added to the first emulsion forming a water-in-oil emulsion. Afterwards, the water-in-emulsion may be cooled to room temperature. Polymeric particles may appear at about 40°C. The aggregation of droplets, which may be referred to as flocculation, may form a matrix with oil droplets that will form pores or conduits in the beads. The particles may be washed with distilled water and alcohol, sized with industrial sieves, and preserved in water.

In Applicant's process, the porous particles are formed by lowering the temperature of the emulsion to "flocculate" the particles. In contrast, Li appears to describe a process of heating an oil-in-water emulsion to form polymeric beads. For example, Li states:

> Once a stable suspension of HIPE microdroplets is obtained, the temperature of the aqueous suspension medium is increased above ambient temperature, and polymerization is initiated. Polymerization conditions vary depending upon the composition of the HIPE. For example, the monomer or monomer mixture and the polymerization initiator(s) are particularly important determinants of polymerization temperature. Furthermore, the conditions must be selected such that a stable suspension can be maintained for the length of time necessary for polymerization. The determination of a suitable polymerization temperature for a given HIPE is within the level of skill in the art. In general, the temperature of the HIPE suspension should not be elevated above 85.degree. C. because high temperatures can cause the suspension to break. Preferably, when AIBN is the oil-soluble initiator and potassium persulfate is the water-soluble initiator, styrene monomers are polymerized by maintaining the suspension at 60.degree. C. overnight (approximately 18 hours).

As such, Applicant submits that Li does not appear to teach or suggest Applicant's claimed method. Furthermore, Applicant submits that Li does not appear to teach or suggest the formation of particles having an average pore size of less than about 1 μm. For example, referring to Table 2 (Col. 23), Applicant submits that most of the particles appear to include "cavities" having a cavity size of about 5-10 μm. Applicant submits that Ex. 10 of the system includes a description of particles having a cavity size of "<2 μm." However, Applicant submits that, without further evidence, Li does not appear to teach that the cavities are less than about 1 μm. As such, Applicant submits that the combination of Freiburghaus and Li does not appear to teach or suggest all of the features of Applicant's claims.

G. The Claims Are Not Obvious Over Freiburghaus in view of Kambara et al.

The Written Opinion states that claims 65-68, 70, and 71 lack an inventive step as being obvious over Freiburghaus et al in view of U.S. Patent Application No. 6,288,220 to Kambara et al. ("Kambara"). Applicant respectfully disagrees that the claims lack an inventive step.

The Written Opinion states

> [w]hile the reference of Freiburghaus discloses the use of analyte particles, the reference is silent as the method and device recited in claims 65-68, 70, and 71 above. The reference of Kambara et al. discloses that it is known in the art to employ probe particles in a flow and capture system that optically images the analyte particles.

Claim 65, 67, and 68 include a combination of features including, but not limited to, the features of "wherein the porous particle comprises a plurality of pores having a diameter of less than about 1 μm."

Applicant submits that for at least the reasons previously mentioned Freiburghaus does not appear to teach or suggest at least the quoted features. Applicant further submits that Kambara does not appear to teach or suggest at least the feature of a "porous particle comprises a plurality of pores having a diameter of less than about 1 μm." Applicant submits that claim 65, 67 and 68 and any dependent claims thereof are patentable over Freiburghaus in view of Kamabara.

H. Remarks

The Written Opinion stated that claims 50-55 and 69 were novel, had an inventive step, and had industrial applicability. The Written Opinion stated that "[c]laims 50-55 and 69 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest using a plurality of wavelengths and/or masks as recited in the above claims to form an image of the collected image of the collected material on a collection membrane." Applicant submits that claims 50-55 and 69 are patentable for at least the reasons cited in the Written Opinion.

H. Summary

Applicant invites this Authority to contact the undersigned if it deems necessary to do so.

Should any fees be required for any reason relating to the enclosed materials, please deduct said fees from Meyertons, Hood, Kivlin, Kowert & Goetzel, P.C. Deposit Account No. 50-1505/5119-11101/EBM.

Please date stamp and return the enclosed postcard to acknowledge receipt of these materials.

Respectfully submitted,

MEYERTONS, Eric B., Attorney for Applicants
Reg. No. 34,876

Meyertons, Hood, Kivlin, Kowert & Goetzel, P.C.
P.O. Box 398
Austin, Texas 78767-0398
(512) 853-8800 telephone
(512) 853-8801 facsimile

Date: April 26, 2004

forming a plurality of particles, wherein each particle comprises a receptor coupled to a polymeric resin, wherein a plurality of different receptors are coupled to the particles;

interacting the plurality of particles with an analyte;

determining which particles interact with the analyte and the extent to which the interact with the analyte;

separating particles that interact with the analyte and meet a predetermined criteria from particles that do not substantially interact with the analyte or do not meet a predetermined criteria;

adding the separated particles that interact with the analyte and meet the predetermined criteria to a sensor array.

71. The method of claim 70, wherein separating the particles comprises separating the particles using a flow cytometer.

72. An analyte detection device comprising:

a body;

a porous membrane coupled to the body.

Serial No. 09/775,343 entitled "Portable Sensor Array System"; and U.S. Patent Application Serial No. 10/072,800 entitled "Method and Apparatus for the Confinement of Materials in a Micromachined Chemical Sensor Array".

Method of Testing for Microbes Using A Membrane System

In another embodiment, a membrane based flow sensor was prepared which is configured to accommodate the capture of microbes with a filter placed within the fluidics device. Microbes, whose size is larger than the pores of the filter, are captured in the flow cell assembly. The captured microbes may be analyzed directly or may be treated with visualization compounds.

A variety of microbes may be captured and analyzed using a membrane based flow sensor as described herein. As used herein, "microbe" refers to any microorganism, including but not limited to, a bacteria, spore, protozoan, yeast, virus, and algae. Some microbes that are of particular interested for detection include a variety of toxic bacteria. Examples of bacteria that may be detected using a membrane based flow sensor include, but are not limited to *Escherichia coli O157:H7, Cryptosporidium, Vibrio cholerae, Shigella, Legionnella, Lysteria, Bacillus globigii,* and *Bacillus anthracis* (anthrax). Viruses may also be detected using a membrane.

Shown in FIG. 1 is an exploded view of a membrane based flow sensor 100. Flow sensor 100 includes a membrane 110 that is sandwiched between at least two members 140 and 150. Members 140 and 150 are configured to allow fluid to flow to and through membrane 110. Members 140 and 150 are also configured to allow detection of analytes, after the analytes have been captured on membrane 110. A variety of different materials may be used for membrane 110, including, but not limited to, Nuclepore ® track-etched membranes, nitrocellulose, nylon, and cellulose acetate. Generally, the material used for membrane 110 should have resistance to non-specific binding of antibodies and stains used during the visualization and detection processes. Additionally, membrane 110 is composed of a material that is inert to a variety of reagents, buffers, and solvents. Membrane 110 may include a plurality of sub-micron pores that are fairly

After the bead library has been optimized for the indicator, the beads that have been collected represent a reduced population of the originally produced beads. If the population of beads is too large, additional screening may be done by raising the intensity threshold. Now that the beads that exhibit optimal interaction with a receptor have been identified, the remaining beads are optimized for displacement of the indicator by the analyte of interest. Thus, the remaining beads are treated with a fluid that includes the analyte of interested, as depicted in FIG. 11C. The analyte is represented by the circle. For some beads, the analyte will cause displacement of the indicator, causing the color or fluorescence of the bead to be reduced, as depicted in FIG. 11D. The intensity of the color or fluorescence of the bead after it interacts with an analyte will be based on how the competitive displacement of the indicator. A bead that exhibits weak or no color or fluorescence when treated with an analyte is the most desirable. Such beads show that the analyte is readily bound by the receptor and can readily displace the indicator from the receptor.

Once again a flow cytometer may be used to determine the optimal beads for use in an assay. A library of beads that have been optimized for interaction with an indicator are treated with a fluid that includes an analyte. The treated beads are passed through a flow cytometer and the beads are separated based on intensity of color or fluorescence. The beads that exhibit a color or fluorescence below a predetermined intensity are collected, while beads that show a color or fluorescence above the predetermined intensity are sent to a waste collection. The collected beads represent the optimal beads for use with the selected analyte and indicator. The identity of the receptor coupled to the bead may be determined using known techniques. After the receptor is identified, the bead may be reproduced and used for analysis of samples.

This page intentionally left blank

This page intentionally left blank

This page intentionally left blank

This page intentionally left blank

Serial No. 09/775,343 entitled "Portable Sensor Array System"; and U.S. Patent Application Serial No. 10/072,800 entitled "Method and Apparatus for the Confinement of Materials in a Micromachined Chemical Sensor Array".

Method of Testing for Microbes Using A Membrane System

In another embodiment, a membrane based flow sensor was prepared which is configured to accommodate the capture of microbes with a filter placed within the fluidics device. Microbes, whose size is larger than the pores of the filter, are captured in the flow cell assembly. The captured microbes may be analyzed directly or may be treated with visualization compounds.

A variety of microbes may be captured and analyzed using a membrane based flow sensor as described herein. As used herein, "microbe" refers to any microorganism, including but not limited to, a bacteria, spore, protozoan, yeast, virus, and algae. Some microbes that are of particular interested for detection include a variety of toxic bacteria. Examples of bacteria that may be detected using a membrane based flow sensor include, but are not limited to *Escherichia coli O157:H7, Cryptosporidium, Vibrio cholerae, Shigella, Legionnella, Lysteria, Bacillus globigii,* and *Bacillus anthracis* (anthrax). Viruses may also be detected using a membrane.

Shown in FIG. 1 is an exploded view of a membrane based flow sensor 100. Flow sensor 100 includes a membrane 110 that is sandwiched between at least two members 140 and 150. Members 140 and 150 are configured to allow fluid to flow to and through membrane 110. Members 140 and 150 are also configured to allow detection of analytes, after the analytes have been captured on membrane 110. A variety of different materials may be used for membrane 110, including, but not limited to, Nuclepore ® track-etched membranes, nitrocellulose, nylon, and cellulose acetate. Generally, the material used for membrane 110 should have resistance to non-specific binding of antibodies and stains used during the visualization and detection processes. Additionally, membrane 110 is composed of a material that is inert to a variety of reagents, buffers, and solvents. Membrane 110 may include a plurality of sub-micron pores that are fairly

After the bead library has been optimized for the indicator, the beads that have been collected represent a reduced population of the originally produced beads. If the population of beads is too large, additional screening may be done by raising the intensity threshold. Now that the beads that exhibit optimal interaction with a receptor have been identified, the remaining beads are optimized for displacement of the indicator by the analyte of interest. Thus, the remaining beads are treated with a fluid that includes the analyte of interested, as depicted in FIG. 11C. The analyte is represented by the circle. For some beads, the analyte will cause displacement of the indicator, causing the color or fluorescence of the bead to be reduced, as depicted in FIG. 11D. The intensity of the color or fluorescence of the bead after it interacts with an analyte will be based on how the competitive displacement of the indicator. A bead that exhibits weak or no color or fluorescence when treated with an analyte is the most desirable. Such beads show that the analyte is readily bound by the receptor and can readily displace the indicator from the receptor.

Once again a flow cytometer may be used to determine the optimal beads for use in an assay. A library of beads that have been optimized for interaction with an indicator are treated with a fluid that includes an analyte. The treated beads are passed through a flow cytometer and the beads are separated based on intensity of color or fluorescence. The beads that exhibit a color or fluorescence below a predetermined intensity are collected, while beads that show a color or fluorescence above the predetermined intensity are sent to a waste collection. The collected beads represent the optimal beads for use with the selected analyte and indicator. The identity of the receptor coupled to the bead may be determined using known techniques. After the receptor is identified, the bead may be reproduced and used for analysis of samples.

~~**HIV detection**~~

~~More than 35 million HIV-infected people live in developing countries with significant resource limitations. Although effective antiretroviral therapy has been available in developed countries for almost a decade, fewer than 300,000 people living in developing countries are believed to be receiving treatment. One major obstacle, the cost of antiretroviral medications~~

(ARVs), is being addressed by price reductions and the advent of generic versions of ARVs. A second obstacle, the cost and technical requirements of the sophisticated laboratory tests used to initiate and monitor HIV treatment, remains to be addressed.

Of particular importance, measurements of CD4+ T lymphocytes are essential for evaluating HIV infected patients. CD4 counts, expressed as either absolute numbers of CD4 cells per milliliter of blood, a percentage of total T lymphocytes, or as the ratio of CD4:CD8 T lymphocytes, have enormous prognostic and therapeutic implications, and form the basis for most treatment decisions. In developed countries, CD4 counts are performed using flow cytometers, which use lasers to excite fluorescent antibody probes specific for CD4 and other T cell markers. The emitted light is collected by a series of photomultiplier tubes, and these signals are analyzed to differentiate CD4+ T cells from CD8+ T cells and other cellular components of blood. Costs for flow cytometers range from $30,000 to $100,000 per machine. This expense, as well as their need for high wattage electricity, regular maintenance and costly reagents, makes flow cytometers impractical, and unsustainable in resource scarce settings.

Several preliminary efforts have been made to develop alternative, affordable CD4 counting methods for resource poor settings. Single purpose flow cytometers for CD4 counting have been developed, but remain costly. A second approach has been to use low cost immunomagnetic separation of CD4 cells from other blood cells, followed by standard cell counting methods using a light microscope. While significantly cheaper, these methods are low throughput and less accurate than the flow cytometry based methods.

To address the significant need for low cost CD4 counts for resource poor settings, we have applied recent advances in microdetection technologies to the development of accurate, affordable and portable CD4 counts. Described herein is an affordable microchip based CD4 assay, including basic performance characteristics of a prototype version and preliminary evaluations in HIV infected and control subjects.

~~A microporous lymphocyte capture membrane was used in a membrane based flow sensor as previously described. For preliminary studies, CD4 cells were purified by immunomagnetic separation from buffy coats obtained from healthy donors. All CD4 preparations were greater than 98% pure by flow cytometric analysis. CD4 cells labeled with Alexa488 conjugated anti CD4 antibodies were introduced to the flow cell in amounts ranging from 0 to 200,000 cells in 1 ml of cell media, and washed with 2 to 5 milliliters of phosphate buffered saline (PBS).~~

~~For studies on human subjects, 20 microliters of whole blood obtained by venipuncture were incubated with 2 microliters of Alexa488- or Alexa647 conjugated antibodies to CD3, CD4 and/or CD8. After 8 minutes, the sample was introduced directly to the flow chamber without further processing, and then washed with 2 milliliters of PBS. Images of stained cells captured in the microchamber were then obtained and processed for analysis. For some experiments, after image collection 2% glutaraldehyde was introduced into the flow cell and the cells fixed for scanning electron microscopy.~~

~~The membrane based flow sensor was immobilized on the stage of a microscope system equipped with a medium pressure mercury lamp as a light source. For each study subject, a total of five non overlapping regions of the lymphocyte capture membrane in the floor of the microchamber were imaged using a digital camera (DVC, Austin, Texas) attached to the workstation. Each region was imaged twice, once under a red wavelength absorption filter (for detection of Alexa 488 fluorescence) and once under a green wavelength absorption filter (for detection of Alexa 647 fluorescence). Except for the preliminary studies, these two images were merged to produce a single image prior to analysis.~~

~~Each image was analyzed using a custom algorithm developed in a commercial image processing software package (Image Pro Plus). Thresholds for red, green and blue intensity were established for optimal definition of lymphocytes against the background, and lymphocytes were characterized by their geometry (size and shape). Cells thus identified were then counted in an automated fashion, with results recorded in a spreadsheet as numbers of CD4CD3-, CD4+CD3+,~~

~~CD8+CD3+, and CD8+CD3- and CD4+CD8+ cells, depending on the combination of antibodies used.~~

~~Blood was obtained by venipuncture from healthy volunteers or HIV-infected subjects at the Massachusetts General Hospital. Samples were processed in parallel by flow cytometry at either the MGH clinical laboratories or at the research facility, using standard 4-color protocols on a FACScalibur flow cytometer. The study was approved by the Institutional Review Board at the Massachusetts General Hospital, and informed consent was obtained from all study subjects.~~

~~Results obtained by the automated microchip method were compared directly with results obtained by flow cytometry for each of the study subjects, and correlated using a standard statistical software package.~~

~~Previously, we developed microchip immunoassays using antibody coupled microbeads immobilized in an inverted pyramidal microchamber. This design has proven extremely effective as a microELISA platform for antigen and antibody detection. For CD4 counting, flow chambers were re-engineered with a floor that consisted of a porous plastic grid, upon which rests a disposable lymphocyte capture membrane filter. These filters have a predetermined pore size ranging from 0.2 to 30 microns. In preliminary studies, pore sizes of between 2 and 5 microns proved optimal for microfluidics of the flow chamber, and for retention of lymphocytes. The unwanted red blood cells and platelets pass through the pores and out of the flow chamber prior to imaging, significantly reducing background fluorescence and improving image quality.~~

~~To determine the correlation between fluorescence light intensity detected in our system and the number of labeled CD4 cells, we added an increasing number of purified CD4+ lymphocytes in 1 mL of RPMI to individual microchambers and measured detectable fluorescence. Individual CD4+ T cells are discernible in the dilute samples. Importantly, there is a linear correlation between the number of cells in the sample and the light intensity when measured from a digital image using a pixel analysis. This established proof of principle that the microchamber and image analysis system could be used to accurately measure and detect populations of lymphocytes labeled with fluorescent markers.~~

~~We next developed an assay to measure CD4 cells directly from whole blood, in a single-step no-lyse system. Using blood obtained by venipuncture from healthy control and HIV-infected subjects, 20 microliters of whole blood were incubated with 2 microliters of fluorescently labeled antibodies to CD3, CD4 or CD8. Based on initial studies of photobleaching and signal intensity, we used only the Alexa line of fluorophores (Molecular Probes) in studies on human subjects. The availability of only Alexa 488 and Alexa 647 conjugated antibodies against CD antigens limited us to two color imaging. After an 8 minute incubation, samples were diluted in 480 microliters of PBS, introduced to the flow chamber, washed with 2 mL PBS, and imaged. The total time from blood processing to image analysis was under 15 minutes.~~

~~Alexa488 conjugated antibodies label the CD4 cells green, and Alexa647 conjugated antibodies to CD3 label all T lymphocytes red. Automatic merging of the images allows the distinction between the CD3+CD4+ T lymphocytes of interest, which appear yellow, the CD4+CD3- monocytes (green), and CD3+CD4- T cells (red). For each study subject, five non-overlapping images are obtained, increasing the size of the sample cell population and improving accuracy.~~

~~Scanning electron micrography of a typical sample prepared as described above and processed inside the microchamber. Unwanted red blood cells are not retained on the filter, but pass through the pores with the saline wash, making lysing unnecessary. This, combined with the high volume wash (100 fold excess for the 20 microliter sample) significantly reduces any background fluorescence and improves the image quality.~~

~~Results for CD4 percentages (number of CD4+CD3+ cells/number of total CD3+ cells) and CD4:CD8 ratios (CD4 percentage/CD8 percentage) obtained from the microchip system were compared with results obtained by flow cytometry for each of the study subjects. Comparisons of the microchip method with flow cytometry for CD4:CD8 ratios gave good correlations.~~